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Toxicity of Synthetic Pyrethroid Lambda-Cyhalothrin and Neem Based Pesticide Neemgold on Zebrafish *Danio rerio* (Cyprinidae)

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Abstract: Pesticides in agricultural run-off affect fish and other aquatic organisms. Fish are common bioindicators of aquatic pollution. In the present study bioassay of a synthetic pyrethroid, Lambda-cyhalothrin and a plant origin natural pesticide, Neemgold was separately done on Zebrafish, *Danio rerio*. The 96h LC_{50} of Lambda-cyhalothrin is 0.119 µg/l and for Neemgold 2.980 µg/l. In this study, Neemgold is less toxic to Zebrafish as compared to Lambda-cyhalothrin because it contains easily biodegradable molecules than the highly persistence broad-spectrum synthetic chemicals. Therefore, use of plant based pesticides is less disastrous and more ecofriendly.

Key wards: *Danio rerio* · Toxicity · Neemgold · Lambda-cyhalothrin

INTRODUCTION

In developing countries like India, pest control programme is conducted on an extensive scale by using synthetic pesticides in view of the occurrence of a large number of pests, with regular periodicity. Increased use of organic pesticides in agriculture, public health and forestry ultimately results in the excess inflow of toxic chemical mainly into the aquatic ecosystem [1]. The aquatic flora and fauna are affected by the toxic substances which eventually enter into their systems or bring about external damages [2, 3]. Several species of fish are susceptible to deleterious effects when exposed to heavy metals, pesticides and other environmental stressors [4].

Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleo-resin extract of genus *Chrysanthemum* flowers [5]. Lambda-cyhalothrin runoff from agricultural fields killed fishes in masses in various part of the world. It is extremely toxic to many aquatic organisms including fish such as the blue gill and lake trout with LC_{50} values less than 1.0 µg/l [6]. It is also toxic to many aquatic invertebrate species [7].

To overcome the hazardous effects of these organic pesticides, recent emphasis is on the use of natural pesticide, which is usually of plant origin. Plants are virtually inexhaustible sources of structurally diverse and biologically active substances [8]. Some plants like neem contain compound of various classes that have insecticidal, piscicidal and molluscicidal properties, unlike synthetic chemical pesticides, which leave harmful residues in the aquatic environment [9, 10]. Botanical insecticides are believed to be more environmentally friendlier because they are easily biodegraded and leave no residues in the environment. Azadirachtin derived from Neem (*Azadirachta indica*, A. Juss), is a very effective and extensively used in various neem based formulations from several years [11]. It has been reported that neem based pesticides are target specific and comparatively less toxic.

However, little work has been done on the toxic effect of neem based pesticides on fish. It is possible to substitute organic pesticides with the plant origin. Fishes are considered indicators of water pollution [12]. The Zebrafish was selected as the test species for toxicological studies according to the recommendation of the International Organization for Standardization [13] and the Organization for Economic Co-operation and Development [14].

The present study was carried out to evaluate comparative effect of a synthetic pyrethroid, Lambda-cyhalothrin and a neem based pesticide, Neemgold on the Zebrafish, *Danio rerio* (Cyprinidae).

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MATERIAL AND METHODS

Zebrafish, *Danio rerio* were reported from Uttar Pradesh [15]. They were collected, stocked and acclimatized in glass aquaria containing dechlorinated water. The water of the aquarium was aerated continuously through stone diffusers connected to mechanical air compressor. Water temperature ranged between 25±2°C and the pH was maintained between 6.6 and 8.5. Fishes were fed twice daily alternately with raw chopped goat liver and brine shrimps. The diet was supplemented with *Drosophila* flies once daily.

For toxicity test Zebrafish of similar age were procured from the laboratory bred general culture. A 96h bioassay was performed in laboratory to determine the 24, 48, 72 and 96h LC_{50} values using five different concentrations of Lambda-cyhalothrin (0.05, 0.10, 0.20, 0.40 and 0.80 μg/l) and five concentrations of Neemgold (0.75, 1.50, 3.00, 6.00 and 12.00 μg/l), previously diluted in acetone. Two replicates of ten fishes for each concentration of pesticides were performed. The stock solution was prepared by serial dilution in acetone. Acetone alone, in the same amount served as control. The randomization of the fish in test aquaria was done, according to the method prescribed by the U.S. Federal Water Pollution Control Administration [16].

The water was changed every 24h and the toxicant was also revised. A fish was considered dead when its gill movements ceased and it did not respond to gentle prodding. Dead fish was removed carefully from the aquaria to avoid deterioration. Lambda-cyhalothrin, with the brand name "Constant" 2.5 % EC of Sree Ramicides Chemicals Pvt. Ltd. Chennai and Neemgold (Azadirachtin 0.03 % m/m and other solvent ingredient neem oil 90.57 %, hydroxy EL 5 %, Epichlorohydrin 0.5 % and Aromex 2.9 %) of Foliage Chemicals Pvt. Ltd. Chennai was purchased from local market.

The results of the bioassay were analyzed by linear regression probit analysis [17] using the StatPlus® version-2008 computer programme. The mortality of exposed Zebrafish was observed at different exposure periods, *viz.* 24, 48, 72 and 96h at different concentrations. The LC_{50} values, 95% Confidence Limits, Slope, Chi-square values were calculated.

RESULTS AND DISCUSSION

After the exposure of both the pesticides, the Zebrafish showed behavioural changes, they aggregated at one corner of the aquarium, swam erratically, the frequency of surface phenomenon becomes greater. The ventilation rate was increased accompanied by loss of equilibrium. Their body colour darkened and the pectoral and pelvic fins got expanded, the fishes rolled vertically prior to death. However, the behavioural changes were more prominent for the synthetic pyrethroid, Lambda-cyhalothrin than that of Neemgold.

The results of the toxic effects are illustrated in table 1. It is evident from the tables that the LC_{50} values decreases with the increase in exposure period. It means that the toxicity of these pesticides increases with the increase in time period. In other word, the mortality of fish was concentration as well as time dependent.

From table 1, it is also evident that Lambda-cyhalothrin is more toxic than Neemgold. The concentration of Lambda-cyhalothrin required for killing the fish is lower than that of the concentration of Neemgold. It was observed that during the exposure of Lambda-cyhalothrin the LC_{50} value after 24h was 1.127 μg/l which decreased to 0.119 μg/l after 96h of exposure. On the other hand, the 24h LC_{50} values of Neemgold was 23.125 μg/l which decreased to 2.890 μg/l after 96h of exposure. Hence, both the pesticides show time-dependent action. The 96h LC_{50} of

Table 1: Toxic effect of Lambda-cyhalothrin and Neemgold pesticides against Zebrafish, Danio rerio (Cyprinidae).

		Confidence Limits			
Treated Period (Hours)	LC50 Values (μg/l)	LCL(μg/l)	UCL (μg/l)	Slope	Chi-square Values
24	1.127*	0.558	12.090	6.60	0.185
	13.135**	9.533	12.182	9.10	0.224
48	0.435	0.261	1.206	7.00	0.241
	9.851	5.595	1 .763	6.66	0.072
72	0.210	0.141	0.317	4.10	0.574
	4.794	3.162	8.644	7.11	0.334
96	0.119	0.079	0.164	3.04	0.056
	2.980	3.068	4.288	6.87	0.192

* Data in upper case belongs to Lambda-cyhalothrin Treatment

** Data in lower case belongs to Neemgold treatment

Lambda-cyhalothrin is 0.119 μg/l whereas for Neemgold is much higher (2.980 μg/l) which indicate the less toxic nature of the plant based pesticide.

The slope value shown in the tables are steep. The LC_{50} values of the pesticide showed a significant ($P<0.05$) negative correlation with exposure time. The Chi-square values were not significant, indicating that the fish population used in the experiments was homogenous.

The Zebrafish behaviour indicated that the fish has adapted to a compensatory mechanism to derive energy during pyrethroid toxicosis [18]. The hyper excitability of the Zebrafish during exposure to Lambda-cyhalothrin may be attributed to the hindrance in the functioning of the enzyme Acetyl Cholinesterase (AChE) in relation of nervous system [19]. It leads to accumulation of AChE which is likely to cause prolonged excitatory post synaptic potential. This may first lead to stimulation and later cause a block in the cholinergic system. It is reported that the 96h LC_{50} of Deltamethrin to *Cyprinus carpio* juveniles was 0.00145 mg/l [20] and reported that 96h LC_{50} of Lambda-cyhalothrin to blue gill sunfish was 0.210 μg/l and for rainbow trout it was 0.240 μg/l [21]. It is reported that the Zebrafish is more sensitive to Deltamethrin with 96h LC_{50} 0.121 μg/l [22] and also Lambda-cyhalothrin was toxic to the embryo of Zebrafish [23].

Although organic pesticides are target specific and effective, but their impact on the environment is mostly deleterious. An organophosphate, Malathion did not cause any delay in the hatching of the eggs however, with increasing concentration the per cent hatching gradually decreased [24]. Plant based pesticides contain active principles with low half life period and their toxic effects on the environment are not too detrimental [25]. However, the toxicity of two neem based pesticides Nimbecidine and Neemgold on a fresh water loach, *Lepidocephalichthys guntea* has been reported [26]. Recently, Achook a neem based pesticide was found toxic to adult Zebrafish [22]. In the present study, the pesticide containing Azadirachtin is less toxic to Zebrafish in comparison to Lambda-cyhalothrin.

To reduce the contamination in the environment it is suggested that use of plant based pesticides should be encouraged [27] because they disintegrate easily into constituent elements without leaving any indelible impression in different regions of the environment [28]. The impact of natural pesticide is much less as compared to the synthetic one (Table 1). It is suggested that more and more plant product should be developed with proper and targeted action, so that, it keeps the environment free from hazardous chemicals. This type of study can also be useful to compare the sensitivity of the various species of aquatic animals and potency of chemicals using LC_{50} values and to derive safe environmental concentration by which there is no lethality and stress to the animals.

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